Campylobacter jejuni in Foods: Its Occurrence, Isolation from Foods, and Injury

ABSTRACT

The following aspects of *Campylobacter jejuni* has been reviewed: characteristics of *C. jejuni*, its occurrence in foods, methods to quantitatively recover the organism from food, and heat injury and freeze-thaw stress of *C. jejuni*. *C. jejuni* can be heat injured in 0.1 M potassium phosphate buffer at 46°C. Heat injury can be demonstrated as the differential count between brucella agar plus ferrous sulfate, sodium metabisulfite and sodium pyruvate (FBP) and brilliant green 2% bile broth agar plus FBP. Heat-injured *C. jejuni* will grow on brucella agar containing either of the three antibiotic mixtures typically used to isolate *C. jejuni*. Heat-injured *C. jejuni* will repair (regain dye and bile tolerance) in brucella broth plus FBP. *C. jejuni* can be freeze-thaw stressed. This stress is demonstrated as a sensitivity to the antibiotic polymyxin B or incubation at 42°C. Addition of succinate and cysteine increased recovery of freeze-thaw stressed *C. jejuni*. Although the presence of injured/stressed *C. jejuni* in foods has not yet been detected, methods are now available to begin this search. The injury/stress process may explain the often encountered difficulty in isolating *C. jejuni*, especially low numbers, from foods.

Campylobacter jejuni has emerged as a pathogen of considerable importance to both food and clinical microbiologists. It is being isolated with increasing frequency from cases of human diarrhea and from foods, often in frequency comparable to *Salmonella (2,6,16,33,36)*. It is found in poultry (*9,17*), red meats (*9,18,31,36*) and raw milk (*9,12,18*). Further, it is the causative agent of human cases of foodborne gastroenteritis associated with the consumption of various foods of animal origin, including raw milk and poultry (*1,33*). Numerous reviews are available on the topic of *C. jejuni*, its characteristics, and its association with foods and human gastroenteritis (*3,5,8,10,26-29,35*).

The association of these organisms with gastroenteritis was first noted by E. O. King (*22*) when she described them as "related vibrios" and indicated that they were distinct from *Vibrio fetus*. Based on their unique biochemical and serological characteristics, G + C mole %, microaerophilic nature, and nonfermentation of carbohydrates, Sebold and Veron (*25*) proposed that these organisms be placed into the genus *Campylobacter* from the Greek meaning "curved rod"

Characteristics of C. jejuni

As indicated above, *C. jejuni* possess certain unique characteristics which warranted creation of a new genus. Some of these characteristics, as well as certain secondary characteristics and other properties and procedures, have been utilized to isolate them from food or clinical specimens. These are listed in Table 1. Because the numbers of *C. jejuni* present in a food are often low and because of the problems of the background flora as well as the fact that the traits and characteristics listed in Table 1 are not exclusive and specific, methods for isolating *C. jejuni* usually rely on a combination of procedures.

Stern (*32*) has evaluated several procedures used to isolate *C. jejuni*. Among the procedures evaluated, he indicated that the method of Doyle and Roman (*13*) was capable of recovering 1 *C. jejuni*/10 g or ml of food in the presence of 10^6 to 10^9 background flora/g or ml of food. Further, the methods were relatively simple to use for food samples. This procedure is presented in Figure 1. Doyle and Roman (*13*) found this selective enrichment procedure useful and sensitive for recovering *C. jejuni* from foods such as raw milk and hamburger, but found that the background flora of chicken skin hampered recovery of campylobacters. Characteristic colonies can tentatively be identified as *C. jejuni* by catalase and oxidase tests, and by examining wet mounts via phase contrast or darkfield microscopy for curved rods exhibiting darting motility. Confirmation is by various biochemical tests including hippurate hydrolysis.

Injury to campylobacters

Microorganisms can be injured/stressed by many of the operational steps involved in food preparation, such as heating, cooling, freezing, acidification/fermentation and chemicals (preservatives, disinfectants) (*7,30*). One of the primary sequelae of injury is the inability of the injured cell to grow on restrictive medium which supports the

TABLE 1. *Some growth properties, procedures, and characteristics of* C. jejuni *that have been used for isolating this bacterium from food and clinical specimens.*

Property, procedure or characteristic	How functions/used (reference)
Growth at 42°C	Enrichment of material at 42°C will suppress background flora, especially with food samples; optimum growth temperature of *C. jejuni*. (*28,29*)
Resistance to specific antibiotics	Suppress the background flora. (*8,27,37*)
Slender rods (0.2 to 0.5 μm wide)	Can filter specimen through a "coarse" (0.65 μm) filter to retain larger background flora. (*37*)
Microaerophilic	Needed for growth of *C. jejuni*, may suppress background flora, especially with foods. (*14,21,28,29*)
Capnophilic (CO_2)	Needed for growth of *C. jejuni*. (*14,21*)
Alkaline peptone water	Represses growth of background flora. (*34*)

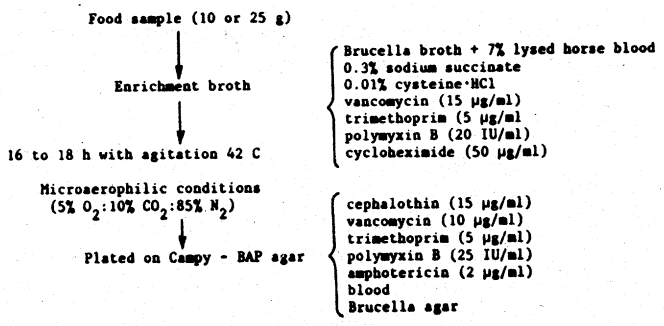

Figure 1. *Isolation of* C. jejuni *from food by the selective enrichment procedure of Doyle and Roman* (13).

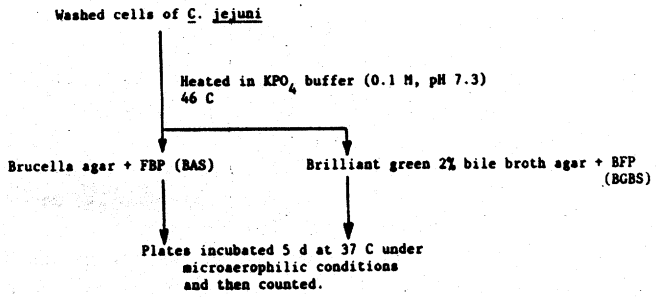

Figure 2. *Procedure for demonstrating and quantitating heat injury in* C. jejuni (23).

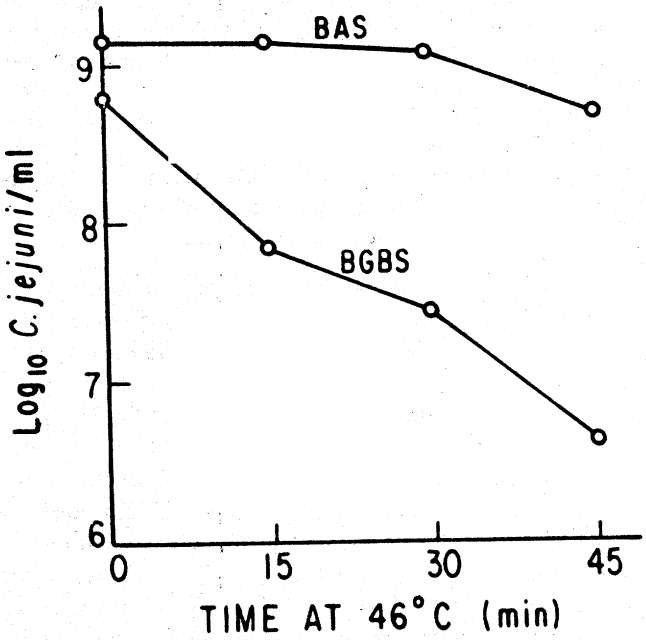

Figure 3. *Effect of plating media on the recovery of* C. jejuni *heated in phosphate buffer at 46°C* (23).

growth of normal/unstressed/uninjured cells. The presence of injured/stressed cells of a particular pathogen (which often go undetected) in a food can give a microbiologist a false estimate of the bacteriological/sanitary quality of a food, and a false picture of the effect of various processing operations on survival of different bacteria.

Injury has been reported for many of the bacteria of interest to the food microbiologist (*7,30*). *C. jejuni* can now be added to this list because of the recent work of Palumbo (*23*) with heat injury, and that of Ray and Johnson (*24*) with freeze-thaw stress.

Palumbo (*23*) recently described a system for demonstrating and quantitating heat injury in *C. jejuni* (Fig. 2). One factor permitting *C. jejuni* to grow on a medium containing brilliant green and bile appears to be inclusion of an aerotolerant supplement (*15;* ferrous sulfate, sodium metabisulfite, and sodium pyruvate; FBP). Tomlinson (*35*) stated that *C. jejuni* would not grow on media typically used for isolation of gram-negative (enteric) bacteria. Palumbo (*23*) found that the inclusion of the FBP mixture supported good growth of *C. jejuni* on both eosin methylene blue (EMB) agar and violet red bile agar compared to media prepared without FBP.

Results of a typical heat injury experiment with *C. jejuni* are shown in Figure 3. Counts on brucella agar supplemented with FBP (BAS) showed relatively little change over the 45-min heating period at 46°C, whereas the count on brilliant green 2% bile broth agar supplemented with FBP (BGBS) showed a dramatic decrease over the same period.

After establishing conditions for injuring *C. jejuni*, it was of interest to determine if the procedures used to isolate *C. jejuni* can recover injured cells. Three antibiotic mixtures added to media used to isolate *C. jejuni* from

food and clinical specimens were added to BAS and the numbers of detectable *C. jejuni* were determined (Table 2). These results indicate that inclusion of these antibiotic mixtures did not interfere with recovery/repair of heat-injured *C. jejuni*.

One characteristic of injured cells is their ability to repair cellular damage and regain the capacity to grow in the presence of selective agent(s). Repair of *C. jejuni* was studied in brucella broth supplemented with FBP and incubated with agitation under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) at 5, 37 and 42°C. Data from the 37°C experiment are shown in Figure 4. These data indicate that repair occurred within 4 h at 37°C. Incubation at 5°C did not allow repair of heat-injured *C. jejuni* over a 5-d period and a gradual decline in viable organisms occurred. *C. jejuni* is characterized by its ability to grow at 42°C, and Doyle and Roman (*13*) took advantage of this by incorporating a 42°C enrichment step in their procedure for isolating *C. jejuni* from foods. Palumbo (*23*) found that heat-injured *C. jejuni* would repair at 42°C within 4 h in brucella broth with FBP under microaerobic conditions. Thus, a 42°C enrichment step would not be contraindicated for use in isolating *C. jejuni* from heat-processed foods.

TABLE 2. *Influence of the addition of antibiotics to BAS on the recovery of* C. jejuni *heated in phosphate buffer at 46°C.*

Plating medium	Log_{10} No. of *C. jejuni* cells at indicated heating time (min)		
	0	20	40
BAS[a]	9.40	9.18	7.90
BGBS[a]	8.96	7.75	5.78
BAS + Butzler[b]	8.28	8.11	7.69
BAS + Skirrow[c]	9.30	9.18	8.00
BAS + Blaser-Wang[d]	9.30	9.18	7.93

[a]See text for compositions of BAS and BGBS.
[b]Butzler antibiotic mixture (Oxoid SR-85; Oxoid Ltd., Basingstoke, England) contained the following: cycloheximide, 0.05 mg/ml; bacitracin, 25 U/ml; colistin sulfate, 10 U/ml; cefazolin sodium, 0.015 mg/ml; and novobiocin, 0.005 mg/ml.
[c]Skirrow antibiotic mixture (Oxoid SR-69; Oxoid) contained the following: vancomycin, 0.01 mg/ml; trimethoprim lactate, 0.005 mg/ml; and polymyxin B, 2.5 IU/ml.
[d]Blaser-Wang antibiotic mixture (Oxoid SR-98; Oxoid) contained the following: vancomycin, 0.01 mg/ml; polymyxin B, 2.5 IU/ml; trimethoprim, 0.005 mg/ml; amphotericin B, 0.002 mg/ml; and cephalothin, 0.015 mg/ml.

It was also observed that *C. jejuni* was extremely sensitive to sodium chloride. This was determined by two separate approaches, i.e., (a) increasing the NaCl concentration in the repair (brucella) broth and (b) increasing the NaCl concentration above the basal medium (0.5%) level. Increasing the NaCl level to 2% in the repair broth proved to be lethal to heat-injured *C. jejuni*. At 1.25% NaCl, repair was inhibited. Increasing the NaCl level in BAS in attempting to develop a selective plating medium to quantitate heat-injured *C. jejuni* also inhibited *C. jejuni*. These observations were supported by the findings of Doyle and Roman (*11*) who reported that as little as 1% NaCl retarded growth of *C. jejuni* and was contraindicated for use in media used to recover or enumerate this organism.

Ray and Johnson (*24*) described an indirect procedure which suggested that *C. jejuni* is stressed (injured/damaged) by freezing and thawing. Lacking a direct plating system, they found that *C. jejuni* became sensitive to antibiotics after freezing and thawing (Fig. 5). Because antibiotic mixtures are usually used in the isolation of *C. jejuni*, they studied this sensitivity further and found that freeze-thaw stress made *C. jejuni* sensitive to polymyxin B (Fig. 6). In addition to the antibiotic repressing growth of the freeze-thaw stressed *C. jejuni*, incubation at 42°C also caused a somewhat greater loss of viability than did incubation at 37°C.

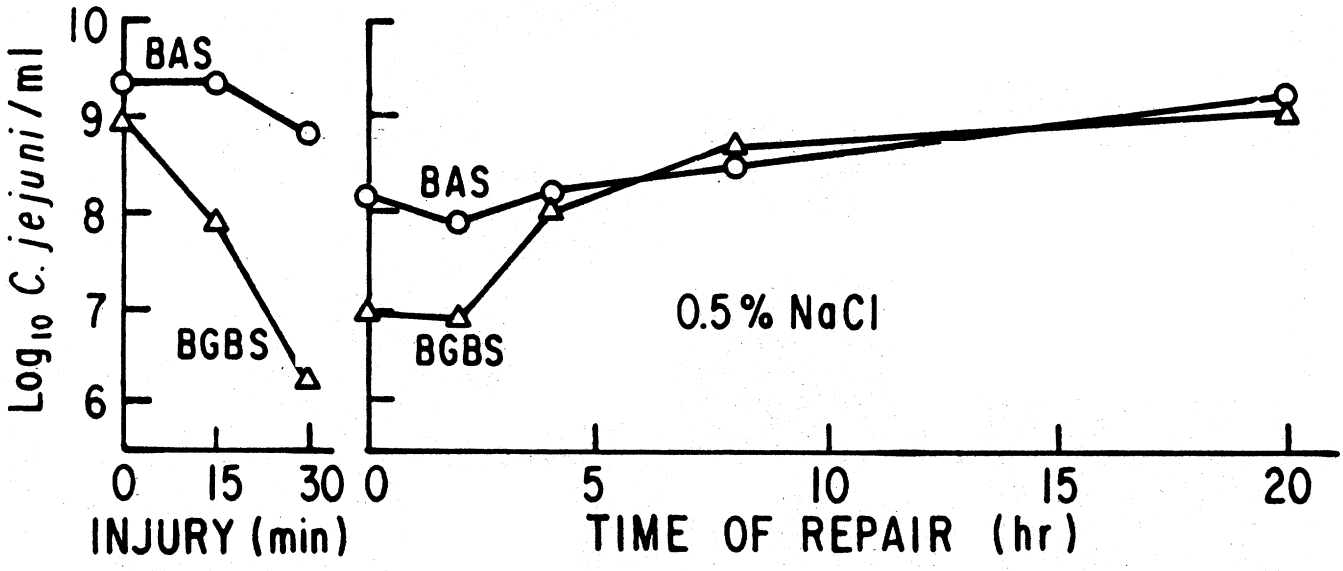

Figure 4. *Repair of heat-injured* C. jejuni *in brucella broth at 37°C* (23).

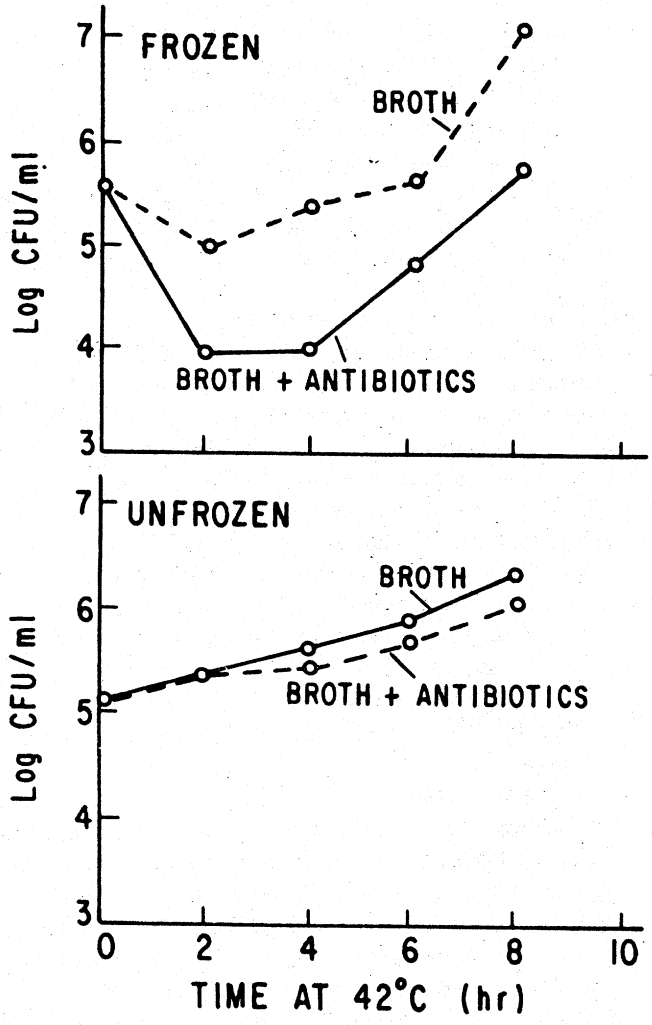

Figure 5. *Growth of* C. jejuni *at 42°C in brucella broth with and without antibiotics. Cells not frozen are compared with cells frozen* (24).

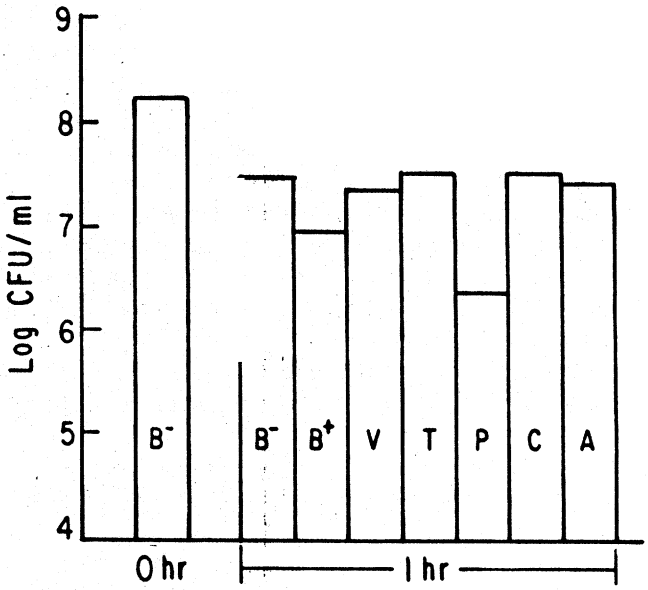

Figure 6. *Effect of antibiotics in brucella broth on survival of freeze-thaw stressed* C. jejuni *at 42°C* (24). *T = brucella broth plus trimethoprim (5 mg/L); P = brucella broth plus polymyxin (2500 IU/L); C = brucella broth plus cephalothin (15 mg/L); B⁻ = brucella broth without antibiotic mixture; B⁺ = brucella broth with antibiotic mixture; V = brucella broth plus vancomycin (10 mg/L); and A = brucella broth plus amphotericin (2 mg/L).*

Because both nutrients and peroxide scavengers can affect recovery of injured cells (*24*), Ray and Johnson studied what effect adding sodium succinate (0.3%) and cysteine•HCl (0.01%) (SCy) to brucella broth had on recovery of freeze-thaw stressed *C. jejuni*. Adding these supplements allowed as few as 10 freeze-thaw survivors per ml to grow (to visible turbidity), whereas thiodipropionic acid and FBP did not. They determined that the addition of SCy, a delay in the addition of the polymyxin B alone or the antibiotic mixture containing it, or a preliminary incubation at 37°C favored repair of freeze-stressed *C. jejuni*. Based on their studies, they recommended the following procedure for maximizing the isolation of *C. jejuni* from frozen foods: (a) a preliminary incubation of the food sample in brucella broth supplemented with SCy, the four antibiotics (vancomycin, trimethoprim, cephalothin and amphotericin) under microaerobic conditions for 6 h at 37°C, followed by the addition of polymyxin B and incubation under microaerobic conditions for an additional 24 h at 42°C.

Freeze stress in *C. jejuni* has also been studied by Humphrey (*20*). His observations were similar to those reported by Ray and Johnson (*24*) except that his strain of *C. jejuni* was sensitive to rifampicin instead of polymyxin B after freeze-stress. Further work is needed to clarify which antibiotics are contraindicated in recovering freeze-stressed *C. jejuni*.

Some characteristics of heat-injured and freeze-thaw stressed *C. jejuni* are presented in Table 3. Because the two reports on which this table is based are the first for this organism and are very recent, it has not been possible to use these procedures to determine if food products contain injured/stressed *C. jejuni* which can be recovered and quantitated. These two reports provide the basis of examining processed food for injured/stressed *C. jejuni*. The presence of injured/stressed *C. jejuni* may be inferred from the difficulties often encountered in isolating *C. jejuni*. Investigators have even incorporated steps which subsequently were found to favor recovery of stressed *C. jejuni*. For example, Doyle and Roman (*13*) added the SCy mixture to their enrichment broth for the isolation of *C. jejuni* from refrigerated foods.

Because of the organism's unique physiology, it often cannot be determined whether any of the specific procedures recover normal and/or stressed cells. *C. jejuni* is extremely sensitive to oxygen levels found in air (20%), hence the use of microaerobic conditions (5% O_2) is commonly employed. Smibert (*29*) has indicated that with large inocula, microaerobic conditions are not as critical as with small inocula. Thus, Heisick et al. (*19*) have reported that continuous gassing with the microaerobic gas mixture increased recovery of low numbers

TABLE 3. *Comparison of heat-injured and freeze-thaw stressed* C. jejuni.

Type of injury	Damage indicated by sensitivity to	Sensitivity to antibiotics	Repair at 42°C	Nutrient supplement useful	Repair sensitive to NaCl
Heat injured	Brilliant green and bile	No	Yes	No	Yes at 1.25%
Freeze-thaw stressed	High temperature of incubation and polymyxin B or rifampicin	Yes	No	Yes succinate and cysteine•HCl	Not tested

of *C. jejuni* from food (ground beef). Further, it is known for *Staphylococcus aureus,* that heat injury causes a decrease in catalase activity (*4*). Palumbo (*23*) did not investigate the effect of heat on catalase activity in *C. jejuni*. Smibert (*28*) has reported that *C. jejuni* is extremely sensitive to H_2O_2.

Similar to detection of other foodborne pathogens, the presence of injured *C. jejuni* must be taken into account. The inability to recover *C. jejuni* in food may be due to the presence of injured *C. jejuni*. The organism is quite sensitive to superoxide and peroxides formed when light and heat react with brucella broth. Because foods contain the same classes of compounds as brucella broth and are also exposed to light and heat during processing, various superoxides and peroxides are likely formed in the food, and could injure and ultimately inhibit the growth of *C. jejuni*. Nonfermentative organisms are generally acid sensitive, hence it is possible that *C. jejuni* is sensitive to low pH values and can be readily acid injured. It may ultimately be determined that recovery methods for *C. jejuni* may have to be tailored specifically for specific classes of foods, taking into account how the processing and composition of the food may have injured/damaged *C. jejuni,* and how the method must be modified to allow repair and recovery of as many *C. jejuni* as possible.

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